ORIGINAL RESEARCH PAPER

Volume-7 | Issue-2 | February-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

ASSESSMENT OF SERUM LUTEINIZING HORMONE, FOLLICLE-STIMULATING HORMONE AND PROLACTIN LEVELS IN POSTMENOPAUSAL WOMEN FROM TERTIARY CARE HOSPITALS OF PUNJAB

Biochemistry

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ABSTRACT

Background: Menopause is the natural process of ageing during which a woman passes from reproductive to non reproductive phase with cessation of cyclic ovarian functions as manifested by cyclic menstruation.

Aim The aim of this study was to assess circulating levels of gonadotropins like luteinizing hormone, follicle-stimulating hormone and prolactin as representative of pituitary hormones in postmenopausal women in comparison to premenopausal women from tertiary care hospitals of Punjab.

Material and Methods: 40 premenopausal women and 40 postmenopausal women were included in the study. Fasting blood samples were collected for the assessment of luteinizing hormone, follicle-stimulating hormone and prolactin levels in both premenopausal and postmenopausal women.

Results: A significant increased was recorded in serum luteinizing hormone, follicle-stimulating hormone and prolactin levels in postmenopausal women in comparison to premenopausal women

Conclusion: A significant increase in luteinizing hormone, follicle-stimulating hormone and prolactin levels in postmenopausal women suggested that we should evaluate thyroid functions along with hormonal evaluation to prevent further complications in postmenopausal women.

KEYWORDS

Premenopause, Postmenopause, Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH) and Prolactin.

INTRODUCTION

Menopause is the natural process of ageing during which a woman passes from reproductive to non reproductive phase with cessation of cyclic ovarian functions as manifested by cyclic menstruation. The menopause is a complex situation determined by multiple endocrine, psychosexual, and cultural factors/ with the possible influence of biological, nutritional, and environmental factors[1] At variance with the menarch, no secular trend has been demonstrated for menopause, which seems unchanged since the middle ages[2]. The transition from reproductive to non-reproductive phase is the result of a major reduction in estrogen production and large number of hormonal changes by the ovaries[3]. Some reports, however, claim that smokers and vegetarians have earlier menopause[4,5] Serum luteinizing hormone (LH) and folliclestimulating hormone (FSH) increase as a consequence of decreasing ovarian feedback elements. Follicle-stimulating hormone increase precedes that of LH during the menopausal transition[6] and both show a rapid oscillatory pattern[7] Chakravarti et al.[8] found a decrease in FSH levels in advanced menopause. However, there is scarce information about the epidemiological profile of gonadotropins and prolactin (PRL) secretion during perimenopausal years.

In our previous study[9], we have found a significant alterations in the levels of T_3, T_4& TSH levels in postmenopausal women with respect to pre menopausal women and are more prone to thyroid disorders could be responsible for impaired fertility etc.

So, in the present work, we studied the variations in gonadotropins representative of pituitary hormones like luteinizing hormone, follicle-stimulating hormone and prolactin in postmenopausal women in comparison to premenopausal women from tertiary care hospitals of Punjab.

MATERIAL AND METHODS

The present case-control study was carried out in the Department of Biochemistry, Government Medical College - Patiala in collaboration with Department of Obstetrics and Gynaecology, Rajindra Hospital Patiala on 80 subjects in the age range of 25 - 70 years. These subjects were taken from general population attending the outdoor patients of Department of Obstetrics and Gynaecology Rajindra Hospital Patiala and were divided into following two groups based on their menopausal status.

Group- 1: 40 premenopausal women with age range of 25 - 45 years
Group-2: 40 postmenopausal women in the age range of 50 - 70 years.

These subjects were recruited from rural and as well as from urban areas of Patiala District of Punjab state.

Ethical Issues: The study protocol was approved by the institutional ethic committee. Study details & potential risks and benefits were explained to individuals taking part in the study and at least one attendant. A written informed consent was obtained voluntarily from the subjects before entering into the study.

Inclusion Criteria

- Premenopausal women in the age group of 25-45 years
- Postmenopausal women in the age group of 50-70 years with natural menopause

All subjects recruited for the study were vegetarian and there was no positive family history of CVD, thyroid dysfunction, Diabetes, Kidney disease etc, in these subjects. These subjects were interviewed by questionnaire regarding the detailed information on their lifestyle, medical history, diet etc.

Exclusion Criteria

- Age less than 25 years and more than 70 years
- Pregnancy
- Abnormal uterine bleeding
- Surgical menopause
- Hypertension, Diabetes Mellitus, Thyroid disorders
- Hepatic disease
- Acute illness
- Patients on lipid lowering medication, patients on HRT.

Measurements of Anthropometric Parameters

The examination of body weight was done by taking weight in kilogram (kg) and height was measured in centimeters. The BMI was calculated from the formula: BMI = weight in kg/(height in meters squared). A complete lipid profile, fasting and postprandial glucose levels, and Blood pressure (systolic and diasystolic) were carried out in adolescents, who entered the study as per a predesigned performa for assessing the signs of chronic heart failure, diabetes and also the presence of any exclusion criteria.

Collection and processing of blood sample:

Fasting (12 hours fasting) blood sample (approximately 5 ml) were collected plain vacutainer from both the groups (Premenopausal and Postmenopausal women subjects) and Plain vacutainer was kept at 37°C for 20 min and then centrifuged at 3000rpm for 15 minutes. A clear supernatant (serum) was used for the estimation of LH, FSH and

Prolactin assays.

i.Determination of Follicle Stimulating Hormone (FSH): FSH levels in the serum of postmenopausal and premenopausal women were determined by using commercial available standarized kits manufactured by Transasia Biomedical Private Limited, Mumbai (India);

PRINCIPLE:
In this method, standards, patient specimens and /or controls (containing the native FSH antigen) are first added to streptavidin coated wells. Biotinylated monoclonal and enzyme labeled antibodies are then added and the reactants mixed: these antibodies have high affinity and specificity and are directed against distinct and different epitopes of FSH.[10]

Reaction between the various FSH antibodies and native FSH occurs in the microwells without competition or steric hindrance, forming a soluble sandwich complex.

The interaction is illustrated by the following equation:

EnzAb(p) + Ag FSH + BtnAb(m) ⟶ EnzAb(p) - AgFSH - BtnAb(m)

BtnAb(m) = Biotinylated Monoclonal Antibody (Excess Quantity)
AgFSH = Native Antigen (Variable Quantity)
EnzAb(p) – AgFSH - BtnAb(m) = Antigen-Antibodies sandwich complex

Ka = Rate constant of Association
k-a = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

EnzAb(p) - AgFSH - BtnAb(m) + Streotavidin C. W. ⟶ Immobilized complex

Streptavidin C.W. = Streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface.
After equilibrium is attained, the antibody –bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody – bound fraction is directly proportional to the native antigen concentration.

The activity of the enzyme present on the surface of the well is quantities by reaction with a suitable substrate to produce color.

By utilizing several different standards of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.[10]

Reference value:
- Follicular phase : 3- 12mIU/ml
- Midcycle : 8 – 22 mIU/ml
- Luteal phase : 2 – 12 mIU/ml
- Menopausal : 35 - 151 mIU/ml.

ii. Determination of Leutinizing Hormone (LH): LH levels in the serum of postmenopausal and premenopausal women were determined by using commercial available standarized kits manufactured by Transasia Biomedical Private Limited, Mumbai (India).

Principle: In this method, standards, patient specimens and /or controls (containing the native LH antigen) are first added to streptavidin coated wells. Biotinylated monoclonal and enzyme labeled antibodies are then added and the reactants mixed: these antibodies have high affinity and specificity and are directed against distinct and different epitopes of LH.[11]

Reaction between the various LH antibodies and native LH occurs in the microwells without competition or steric hindrance, forming a soluble sandwich complex

The interaction is illustrated by the following equation:

EnzAb(p) + Ag LH + BtnAb(m) ⟶ EnzAb(p)- AgLH- BtnAb(m)

BtnAb(m) = Biotinylated Monoclonal Antibody (Excess Quantity)
AgLH = Native Antigen (Variable Quantity)

EnzAb(p) – AgLH - BtnAb(m) = Antigen - Antibodies sandwich complex

Ka = Rate constant of Association
k - a = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

EnzAb(p) - AgLH - BtnAb(m) + Streotavidin C. W. ⟶ Immobilized complex

Streptavidin C.W. = Streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface.

After equilibrium is attained, the antibody –bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody – bound fraction is directly proportional to the native antigen concentration.

The activity of the enzyme present on the surface of the well is quantities by reaction with a suitable substrate to produce color.

By utilizing several different standards of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.[11]

REFERENCE VALUE:
- Follicular phase : 0.5 – 10.5 mIU/ml
- Midcycle : 18.4 – 61.2 mIU/ml
- Luteal phase : 0.5 – 10.5 mIU/ml
- Menopausal : 8.2 – 40.8 mIU/ml.

iii. Determination of Prolactin: Prolactin levels in the serum of postmenopausal and premenopausal women were determined by using commercial available standarized kits manufactured by Transasia Biomedical Private Limited, Mumbai (India);

Principle: In this method, standards, patient specimens and /or controls (containing the native PRL antigen) are first added to streptavidin coated wells. Biotinylated monoclonal and enzyme labeled antibodies are then added and the reactants mixed: these antibodies have high affinity and specificity and are directed against distinct and different epitopes of PRL.[12]

Reaction between the various PRL antibodies and native PRL occurs in the microwells without competition or steric hindrance, forming a soluble sandwich complex.

The interaction is illustrated by the following equation:

EnzAb(p) + AgPRL + BtnAb(m) ⟶ EnzAb(p) - AgPRL - BtnAb(m)

BtnAb(m) = Biotinylated Monoclonal Antibody (Excess Quantity)
AgPRL = Native Antigen (Variable Quantity)

EnzAb(p) - AgPRL-BtnAb(m)=Antigen-Antibodies sandwich complex

Ka = Rate constant of Association
k-a = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

EnzAb(p) - AgPRL - BtnAb(m) + Streotavidin C.W. ⟶

Immobilized complex

Streptavidin C.W. = Streptavidin immobilized on well

Immobilized complex = sandwich complex bound to the solid surface. After equilibrium is attained, the antibody –bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody – bound fraction is directly proportional to the native antigen concentration.

The activity of the enzyme present on the surface of the well is quantities by reaction with a suitable substrate to produce color.

By utilizing several different standards of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.[12]

REFERENCE VALUE:

- In Premenopausal : 1.2 – 19.5ng/ml
- In Postmenopausal: 1.5 – 18.5ng/ml.

Statistical Analysis:

The data was expressed as Mean ± SD. Differences between the premenopausal and post menopausal women were evaluated using the Student's independent samples "t" test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

A significant ($p \leq 0.01$) increase by 41.92% (from 32.87± 7.438 to 46.65±7.001mIU/ml) was observed in serum LH levels and a significant ($p \leq 0.05$) increase by 22.75% in prolactin (from16.74± 3.597ng/ml to 20.55± 5.985 ng/ml) levels was found in serum prolactin levels of postmenopausal women with respect to premenopausal women (Figure-1). A similar trend of increase by 13.90% (from 131.60± 14.682 mIU/ml to 149.9± 10.637 mIU/ml) was recorded in serum FSH levels of postmenopausal women in comparison to premenopausal women (Figure-1). It is well reported in literature that aging is associated with dramatic changes in gonadotropin secretion in healthy subjects. In women, after the initial elevation of serum gonadotropins that characterizes the menopause, a progressive increase in FSH, LH and prolactin levels occurs with age in the later post-menopausal years. At menopause state, the ovary is no longer respond stimulation by the pituitary gland, therefore, FSH and LH were sustained at elevated concentrations due to lack of negative feedback mechanism from estradiol or inhibin B from the ovary. In 1981, Metcal[13] reported that from ovulatory cycles with low premenopausal levels of FSH, to transient episodes indistinguishable from those found in postmenopausal women with high levels of FSH and LH. Typically, the approach of the menopause was marked by an increased incidence of high postmenopausal levels of FSH and LH.

Yen, 1997[14] revealed that the cessation of follicular development results in drop of oestradiol level causing loss of negative feedback on hypothalamus – pituitary axis which in turn is responsible for increase in levels of FSH and LH by anterior pituitary.

Wang in 2015[15] found that FSH serum concentrations in postmenopausal women with osteoporosis increased notably compared with the control group. The circulating concentration of FSH may play an important role in the acceleration of bone loss in postmenopausal women. FSH increases osteoclastogenesis in vitro.

In our previous study[9], we reported that postmenopausal women are more prone to thyroid disorders by altering the levels of T_3, T_4, TSH. The reports of present study are agreement with the literature reports [18-21] that gonadotropins such as LH, FSH and Prolactin secretions representing pituitary gland are stimulated in postmenopausal women in comparison to premenopausal women.

Conclusion: A significant increase in gonadotropins such as LH, FSH and Prolactin secretions in postmenopausal women suggested that we should evaluate thyroid functions along with hormonal evaluation to prevent further complications in postmenopausal women. Long-term studies with large sample size are required to assess the significance of gonadotropins in postmenopausal women.

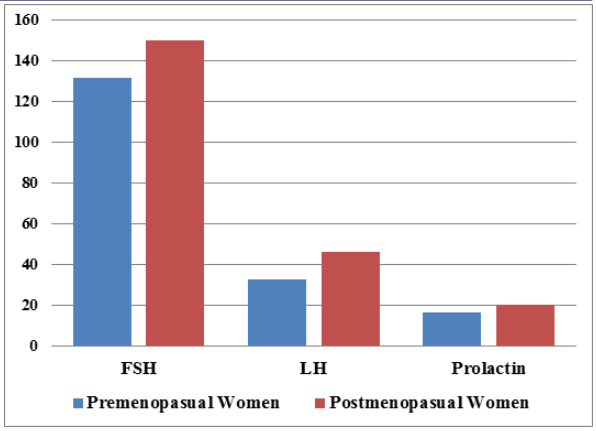

Figure-1: Showing alterations in mean values of 50 observations in Follicle Stimulating Hormone, Leutinizing Hormone and Prolactin levels in premenopausal and postmenopausal women of tertiary care hospitals of Punjab. The values of in Follicle Stimulating Hormone, Leutinizing Hormone in "mIU/ml" and values of Prolactin in "ng/ml".

REFERENCES

1. Mac Mahon B, Worcester J: Age at Menopause. United States 1960-1962 Vital and Health Statistics. Data from the National Health Survey. Series 11(9 NIH 1966.
2. Amundsen DW, Diers CJ: The age of menopause in medieval. Europe. Hum Bio. 1973; l45: 605-609.
3. Adashi EY. The climacteric ovary as a functional gonadotropin driven androgen-producing gland. Fertile Sterile.1994; 62(1): 20-27.
4. Goldin BR, Adlercreutz H, Gorbach SL, W arram JH, Dwyer JH, Swenson L, Woods MN: Estrogen excretion patterns and plasma levels in vegetarian and omnivorous Women. N Eng! J Med 1982; 307: 1542-1551.
5. Jice H, Porter J, Morrison SA. Relation between smoking and age of natural menopause. Lancet 1977; 1: 1354-1356.
6. Sherman BM, West JH, Korenman SG. The menopausal transition: analysis of LH, FSH, estradiol, and progester, one concentrations during menstrual cycles of older women. J Clin Endocrinol Metab. 1976; 42: 639-652.
7. Yen SSC, Tsai CC, Naftolin F, Vandengerb G, Ajabor L: Pulsatile patterns of gonadotropins release in subjects with and without ovarian function. J Clin Endocrinol Metab 1972; 34: 671-679.
8. Chakravarti S, Collins WP, Forecast JD, Newton JR, Oram DH, Studd JW. Hormonal profiles after menopause. Br. Med J 1976; 2: 784-789.
9. Kuldip S, Kaur A, Kaur K, Kaur M. Studies on serum thyroid profile in postmenopausal women from tertiary care hospitals of Punjab. Inter J. Scientific Res. 2018; 7 (1): 2277-8179.
10. Odell WD, Parlow AF. Radioimmunoassay for human follicle stimulating hormone. J Clin Invest,1981; 47: 2551-2565.
11. Kosasa TS. Measurement of Human Luteinizing Hormone. J. Reproduct Med,1981; 2: 201-206.
12. Maddox PR., Jones DL., Mansel RE. Basal prolactin and total lactogenic hormone levels by microbioassay and immunoassay in normal human serum. Acta. Endocrinol. 1991; 125: 621-625.
13. Metcalf MG; Donald RA, Livesey JH. Pituitary-ovarian function in normal women during the menopausal transition. Clin. Endocrinol. (Oxf).1981; 14(3): 245-255.
14. Yen SS. The biology of menopause. Reprod. Med. J .1977; 18: 287- 296.
15. Wang J, Zhang W, Yu C, Zhang X, Zhang H, Guan Q et al. Follicle-Stimulating Hormone Increases the Risk of Postmenopausal Osteoporosis by Stimulating Osteoclast Differentiation. 2015; 4; 108-115.
16. GulabKanwar A, Jain N, Shekawat M, Sharma N. Estimation of LH, FSH, Prolactin and TSH Levels In Polycystic Ovarian Syndrome And Correlation of LH And FSH With Serum TSH Levels. IOSR J.Dental & Medi. Sci. 2015; 14(5); 64-68. DOI: 10.9790/0853-14566468.
17. Graziana C, Concetta C, and Silvia C. FSH and TSH in the Regulation of Bone Mass: The Pituitary/Immune/Bone Axis-review article. Clin. & Develop. Immunol. 2013; Article ID 382698, 6 pages http://dx.doi.org/10.1155/2013/382698.
18. Gary MP, Koenigsberg R,Joseph LZ and Stanley D. Alteration of Serum Pituitary Hormone Levels in Postmenopausal Women With Stroke. Stroke. 1993; 24: 805-808.
19. Anyigor OCS, Onwe PE, Okike PI, Njoba S, Ottah UG., Okorocha AE, and Igwe, JC. Coronary Artery Disease and Menopause: A Consequence of Adverse Lipid Changes. IOSR J.Dental & Medi. Sci. 2015; 14(11): 7-11.
20. Veeresh T, Moulali D, Sarma DVH. A Study on Serum FSH, LH and Prolactin Levels in Women with Thyroid Disorders. Inter. J. Scientific & Res. Pub.2015; 5(3): 1-7.
21. Raj MN, Suresh V, Arun M, Reddy A, Sachan A, Mohan A, VengammaB, Srinivas R. Evaluation of activity of hypothalamo-pituitary-gonadal axis in postmenopausal women suffering from severe acute illness. Ind. J. Med. Res. 2016; 143: 66 – 71.